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Milk protein concentrations in neonatal milk (witch's milk)

P. L. YAP, CHRISTINE L. MIRTLE,* ANN HARVIE† & D. B. L. McCLELLAND‡ *MRC Unit of Reproductive Biology, Chalmers Street, Edinburgh, * University Department of Therapeutics, Royal Infirmary, Edinburgh, † University Department of Child Health, Royal Hospital for Sick Children, Glasgow and ‡ Blood Transfusion Service, Royal Infirmary, Edinburgh, Scotland*

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SUMMARY

Milk protein concentrations were determined in five samples of neonatal milk (witch's milk) by either double antibody radioimmunoassay (IgA) or by single radial immunodiffusion (IgG, lactoferrin, lysozyme and albumin). Neonatal milk IgA concentrations ranged from 0·43 to 118·2 mg/l; the corresponding mean neonatal serum IgA concentration was 2·6±s.e. 1·45 mg/l (n = 48). Sucrose density gradient ultracentrifugation indicated that the IgA detected was of the 11S type. IgG, lactoferrin, lysozyme and albumin were detected in neonatal milk in similar concentrations to those found in maternal milk.

INTRODUCTION

In the mammary gland, increased numbers of plasma cells are observed during pregnancy (Pumphrey, 1977). During the post-partum period, colostrum and milk expressed from the mammary gland contain extremely high concentrations of 11S IgA (McClelland, McGrath & Samson, 1978). Some of the hormonal factors influencing the migration to the mammary gland of precursors of IgA-secreting cells originating from gut-associated lymphoid tissue in the mouse have been elucidated by the work of Weisz-Carrington *et al.* (1978) but little is known about such factors in the human.

Mammary hypertrophy with milk-like secretion from the nipple occurs in some neonates during the first 3 weeks of life. This secretion, sometimes called witch's milk from the historical belief that it was supernatural in origin (Forbes, 1950), and the mammary gland hypertrophy, have been attributed to hormonal changes similar to those which occur in the mother post-partum (Lyons, 1937; Hiba *et al.*, 1977). As IgA can usually be found in external secretions before it is found in serum (Haworth & Dilling, 1966; McKay & Thom, 1969), and as IgA is detectable in low concentrations in neonatal serum (Yap *et al.*, 1979), it was surprising that an earlier study failed to detect IgA in neonatal milk (Hanson, 1961).

We have therefore measured IgA, lactoferrin, lysozyme, IgG and albumin in neonatal milk.

MATERIALS AND METHODS

Five samples of neonatal milk of 50 to 250 μl volume were collected by manual expression from four neonates from 6–28 days post-partum; in one neonate, the neonatal milk was diluted twenty-fold with sodium phosphate buffer to provide a sufficient volume of material for analysis. All samples were stored at −20°C.

A double antibody radioimmunoassay (Yap *et al.*, 1979) was used to measure IgA concentrations. The IgA standard was 11S IgA purified from milk by the method of Newcomb, Normansell & Stanworth (1968).

Single radial immunodiffusion was used to measure concentrations of IgG, lactoferrin, lysozyme and albumin as described previously (McClelland *et al.*, 1978).

A 25–45% sucrose density gradient (236,000 **g** for 3 hr, Sorvall OTD-50) was used to determine the sedimentation coefficient of IgA in a sample of neonatal milk. Fractions of 0·1 ml were collected and stored at −20°C prior to immunoassay without concentration. ^{125}I-7S IgA and ^{125}I-7S IgG were prepared in the same way as ^{125}I-11S IgA and used as markers.

Correspondence: P.L. Yap, Blood Transfusion Service, Royal Infirmary, Edinburgh EH3 9HB, Scotland.

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RESULTS

The binding of ^{125}I-11S IgA to rabbit anti-human IgA was displaced in a parallel manner by the 11S IgA standard and three of the four samples of neonatal colostrum suggesting that immunoassayable 11S IgA was present. The fourth sample was of too small a volume to plot a complete binding curve.

A single peak of immunoassayable IgA was detected by immunoassay of density gradient ultracentrifugation fractions, close to the peak of radioactivity observed for ^{125}I-11S IgA and separated by eight fractions from the peak of radioactivity seen for ^{125}I-7S IgA and ^{125}I-7S IgG (Fig. 1). This confirmed that 11S IgA was present in neonatal milk. IgA levels in neonatal milk using the 11S IgA standard are shown in Table 1. The ratio to neonatal serum IgA levels (Yap *et al.*, 1979) ranged from about 0·16 to 45.

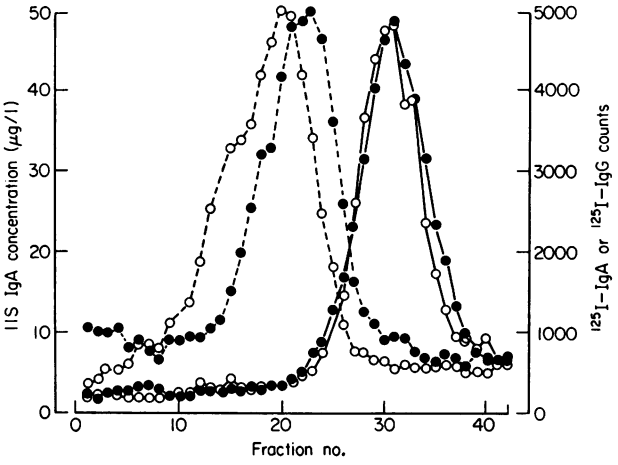

FIG. 1. Sedimentation coefficient determination of a sample of neonatal milk (●---●) by sucrose density gradient ultracentrifugation. Markers consisting of ^{125}I-7S IgA (●———●), ^{125}I-11S IgA (○---○) and ^{125}I-7S IgG (○———○) were also analysed simultaneously in additional identical sucrose gradients; 0·1-ml fractions were collected and assayed by RIA (neonatal milk) or counted in a γ-counter (^{125}I-labelled proteins).

TABLE 1. Milk protein concentrations in neonatal milk

Neonate	Day of collection	IgA (mg/l)	IgG (mg/l)	Lactoferrin (mg/l)	Lysozyme (mg/l)	Albumin (mg/l)
1	6	0·43	100	7050	32	330
	8	0·46	n.d.	i.s.	i.s.	i.s.
2	16	3·25	20	11,200	380	360
3	19	118·17	n.d.	900	n.d.	n.d.
4	28	17·57	80	7000	54	290

n.d. = Not detected.
i.s. = Insufficient secretion for analysis.

IgG, lactoferrin, lysozyme and albumin were detected in the three samples of neonatal milk studied: the concentrations were similar to those in maternal milk collected on the sixth day post-partum (McClelland *et al.*, 1978). The concentrations of IgG and albumin in neonatal milk were very much lower than serum IgG and albumin concentrations at birth (McClelland & Samson, unpublished observations).

DISCUSSION

We found only 11S IgA in neonatal milk and the ratio of neonatal milk IgA levels to neonatal serum IgA

levels is very much higher than for IgG and albumin (McClelland & Samson, unpublished observations). This indicates that the 11S IgA enters neonatal milk by either selective transport of IgA from serum to milk, or local production of 11S IgA. In an earlier analysis by gel filtration of both cord blood and neonatal serum, we have shown that less than 2·5% of the IgA present is 11S (Yap *et al.*, 1979). Our data therefore provides strong evidence for the local synthesis of 11S IgA in the neonatal breast, probably in a similar manner to that occurring in the maternal breast.

Absolute levels of 11S IgA in neonatal milk are approximately a thousand-fold lower than in maternal colostrum and milk. This closely parallels the neonatal serum to maternal serum IgA concentration ratio (Yap *et al.*, 1979), and presumably reflects the immaturity of IgA synthesis in the neonate.

The concentrations of lactoferrin and lysozyme are similar to those found in maternal milk. As these two proteins originate in the glandular acini (Masson *et al.*, 1966) and intralobular ducts (Kraus & Mestecky, 1971) respectively, this finding suggests that neonatal mammary epithelium responds to the hormonal changes of pregnancy and the immediate post-partum period in the same way as maternal mammary epithelium. It is not known whether the high concentrations of lactoferrin and lysozyme are due to epithelial proliferation, increased synthesis by epithelial cells, or both, but the histological appearance of the mammary gland of a full term foetus consists of swollen glandular acini with extensive epithelium, suggesting that epithelial proliferation is occurring (Dabelow, 1957).

In the mouse, it has been suggested that the migration to and maturation within the mammary gland of precursor IgA plasma cells is due to some undefined influence of the mammary epithelium following stimulation by the hormones of pregnancy (Weisz-Carrington *et al.*, 1978). Our data on lactoferrin and lysozyme concentrations suggests that the low absolute concentrations of IgA in neonatal milk may be due to a relative lack of precursor IgA plasma cells rather than to a lack of 'attraction' by mammary epithelium.

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